

## The ultrastructure of articular cartilage of the chicken's knee joint

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**Summary.** *The articular cartilage and synovial membrane of immature and mature chicken knee joints were studied by light, scanning and transmission microscopy. The findings differed from human articular cartilage and we conclude that the chicken knee joint is not suitable as a model for human joint degeneration.*

**Résumé.** *On a étudié par microscopie optique, par microscopie électronique classique et à balayage, le cartilage et la membrane synoviale de l'articulation du genou chez des poulets matures et immatures. Les constatations diffèrent de celles que l'on peut faire chez l'homme. Nous en concluons que le genou du poulet ne peut servir de modèle pour l'étude de la dégénérescence des articulations humaines.*

### Introduction

The articular cartilage of the chicken's knee joint has been used as an experimental model for the induction of arthrosis [17, 18, 19, 20]. We have therefore studied the cartilage by both light and electron microscopy to find out whether it is the same as, or different from, that of mammals. There is relatively little detailed information about the chicken's synovial membrane and we included this in our study.

Mammalian articular cartilage of the knee is typically hyaline [21], but little is known about the ultrastructure of cartilage in birds. It has been noted in chickens that certain regions contain abundant un-

masked collagen fibres resembling fibrous cartilage [18, 20].

### Materials and methods

In this study we used 8 immature chickens and 8 mature White Leghorn hens which were killed by decapitation.

For light microscopy the whole knee joint was immersed in Bouin's fluid and embedded in butyl methacrylate and paraffin [27]. The 7 micron thick sections were stained with HE and alcian blue.

For transmission electron microscopy (TEM), small pieces of articular cartilage from the femoral and tibial condyles and synovial membrane were dissected out and immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours. The specimens were post-fixed overnight in 2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4), dehydrated in acetone and embedded in Epon. Semi-thin sections were stained with Azur II and methylene blue [34] and examined under a light microscope. Ultra-thin sections (Ultracut, Reichert) were contrasted with uranyl acetate and lead citrate, and viewed on a Zeiss M10.

For scanning electron microscopy (SEM), the complete articular cartilages of the femoral and tibial condyles, and the synovial membrane, of immature and mature animals were dissected out without touching them. The specimens were then immersion-fixed (as above) for 48 h, dehydrated in acetone, critical-point dried (Balzers), coated with a sputter coater (Edwards, S 150) and examined with a Novascan 30 Zeiss scanning electron microscope.

### Results

Articular cartilage. Many collagen fibres were seen under the light microscope (Fig. 1). These fibres formed a closely woven network in immature chickens, but in mature birds the distribution of the fibres was different. They were orientated in parallel near the surface but in the deeper zones they ran either in a vertical direction or were arranged in a network extending into the calcified zone next to the underlying

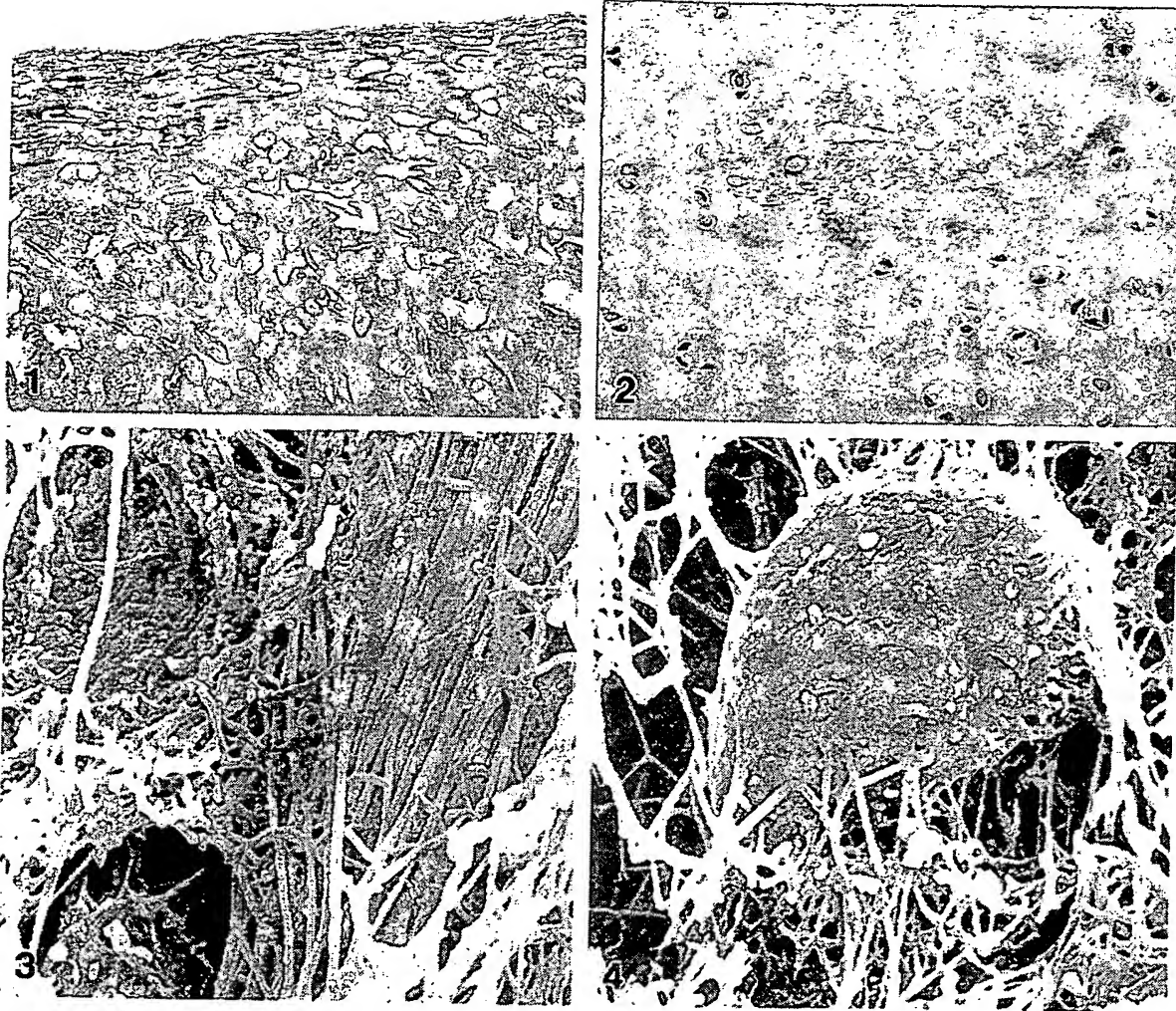


Fig. 1. Light microscopy of chicken femoral articular cartilage. Numerous unmasked collagen fibres are present.  $\times 200$

Fig. 2. Blood vessels are present within the cartilage.  $\times 400$

Fig. 3. SEM of collagen fibrils running at a tangent to the surface of the joint cavity.  $\times 10\,000$

Fig. 4. SEM of a chondrocyte lying in a lacuna and surrounded by a collagenous network.  $\times 10\,000$

ing bone. Most chondrocytes were arranged in pairs and were surrounded by a distinct layer of capsular matrix, even near the bone, and only rarely formed groups of 4 cells in the middle and superficial layers. Near the convex surface, single chondrocytes which lacked a distinct territorial or capsular matrix were seen. A characteristic feature was the presence of blood vessels within the cartilage (Fig. 2).

SEM showed that the articular surface was basically the same in immature and mature specimens

and was absolutely smooth without undulations, humps or pits [14].

At higher magnification the smooth surface was seen to consist of a layer of round matrix granules of varying size, collectively termed the surface coat [1, 14]. No fibrils were found lying between the matrix granules. In immature specimens the surface was interrupted, whereas in mature specimens relatively large polymorphic areas lacking a surface coat were seen. At the bottom of these shallow pits, bundles of

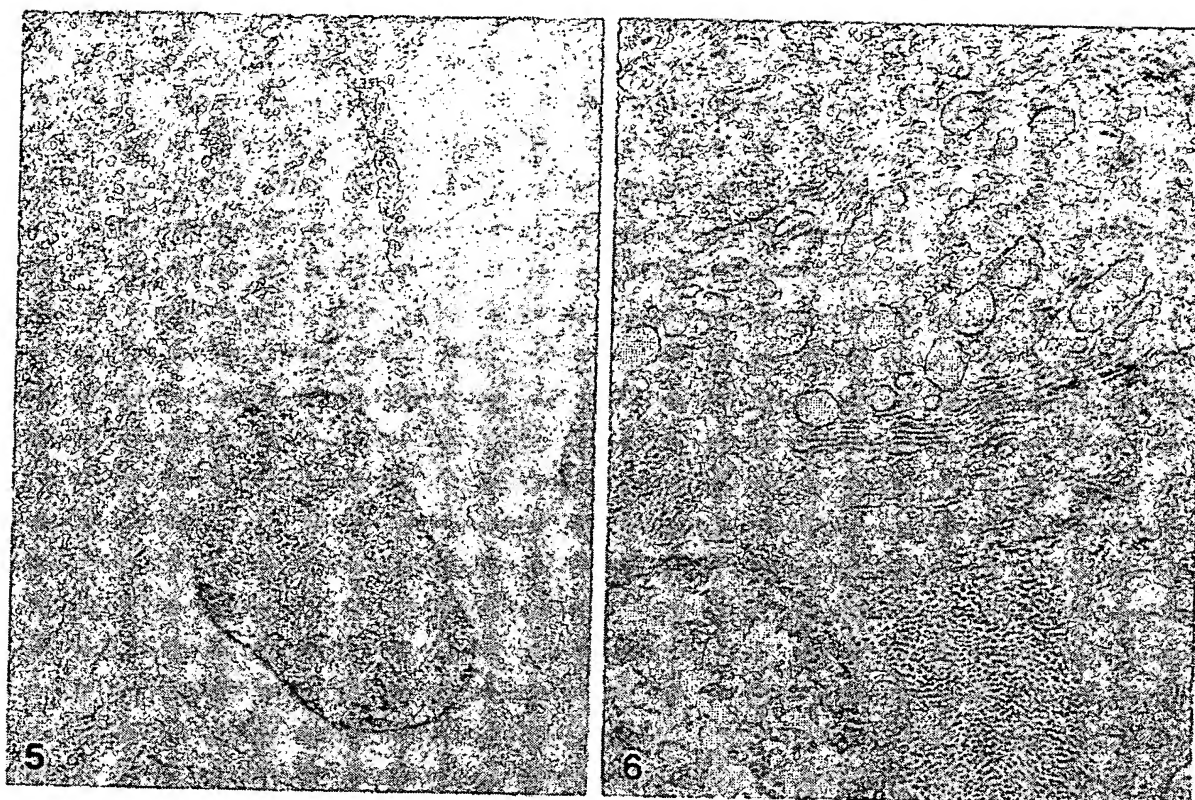


Fig. 5. TEM of a chondrocyte showing a wealth of fine filamentous fibres surrounding the nucleus as a result of which the cells organelles have acquired a peripheral location.  $\times 12\,500$

Fig. 6. A chondrocyte containing well developed Golgi complexes and filaments.  $\times 25\,000$

collagen were seen running parallel to the surface. The surface coat was lacking in the oval zones which clearly differed from the polymorphic zones. These oval zones represented the entrance into deeper cavities surrounded by collagen fibrils arranged in a basket-like fashion. In some instances, the "basket" exhibited gaps and individual chondrocytes surrounded by collagen fibrils together with finely filamentous material could be identified (Figs. 3 and 4). There were abundant intracellular filaments measuring 8–12 nm.

In the immature chickens, there were more filaments in the superficial chondrocytes than in the deeper cells. In mature birds, the cytoplasm of the chondrocytes was often almost entirely filled with these filaments so that only a narrow zone of cytosol, without cytoplasmic organelles, was seen around the nucleus (Fig. 5). Invariably, the usual cell organelles were confined to a thin subplasmalemmal zone. The straight filaments were not randomly orientated, but formed groups of longitudinally or transversely sectioned elements, giving the impression of a densely

knit network. Similar findings were obtained with regard to cytoplasmic organelles and inclusions in chondrocytes of other species.

In immature chickens, the chondrocytes contained a large amount of rough endoplasmic reticulum, free polysomes and a well developed Golgi complex (Fig. 6). Mitochondria were moderately abundant and lipid droplets were scarce.

In mature birds, rough endoplasmic reticulum and polysomes were less plentiful than in immature chickens, and lipid droplets were increased.

The synovial membrane, together with a subsynovial layer, could be clearly distinguished in immature and mature birds. The synovium consisted of from one to three layers of relatively small oval or elongated lining cells, with round or oval eccentrically located nuclei and one or two large nucleoli. The free surface of the lining cells was relatively smooth and did not bulge towards the articular cavity; their cytoplasm appeared homogeneous. The subsynovial layer consisted of loosely arranged connective tissue with a few adipose cells in immature

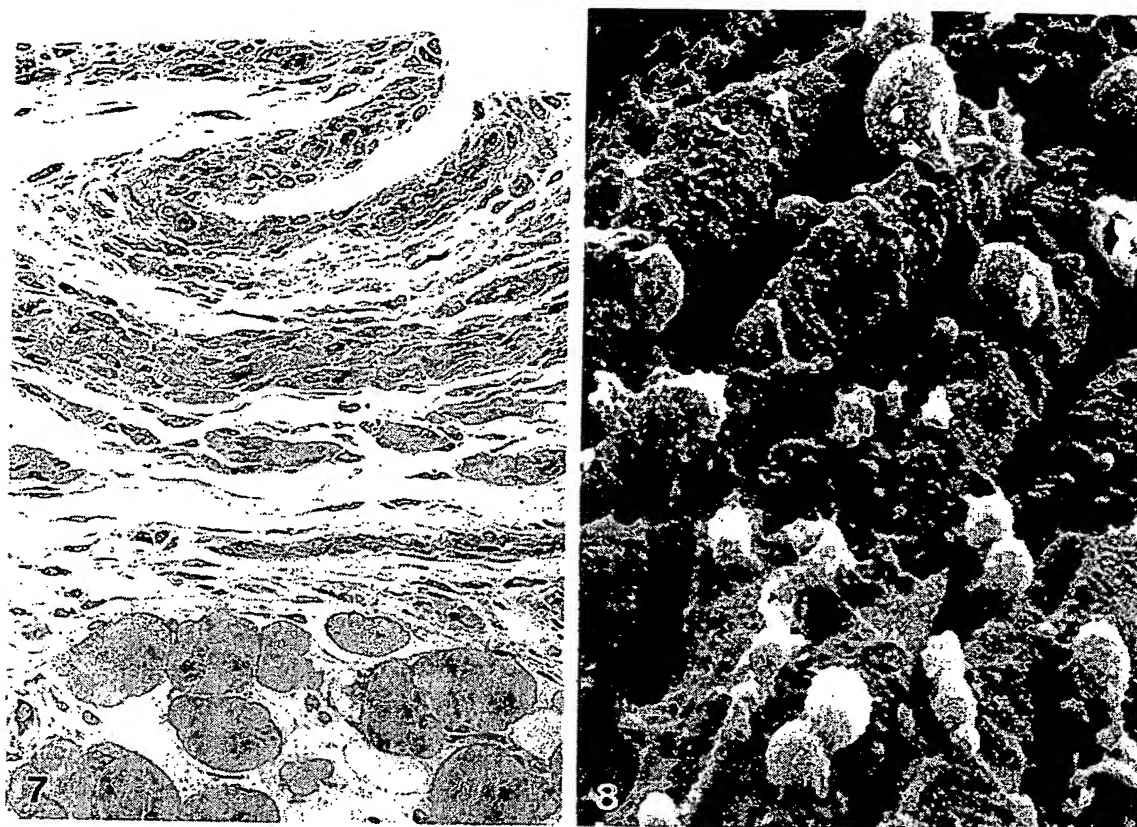


Fig. 7. Semi-thin section showing synovial membrane composed of loosely arranged lining cells and a subsynovial layer.  $\times 200$



Fig. 8. SEM of the intimal layer showing only elongated B cells with some granules on their surface.  $\times 10000$

chickens, but there were large numbers of these cells in mature birds. Blood vessels and nerve fibres were restricted to this zone (Fig. 7).

SEM showed that the synovial membrane in immature and mature birds differed in appearance. In immature specimens, all the cells at the surface of the lining layer appeared elongated and relatively smooth, and were usually arranged parallel to one another. The gaps between the lining cells were filled with delicate fibrils which formed what looked like intercellular bridges (Fig. 8).

In mature birds, two types of lining cells were differentiated. One had relatively smooth contours and a few cytoplasmic processes while the other was characterised by numerous irregular protrusions, giving the cell a cauliflower-like appearance [8] which was seen to bear microvilli under higher magnification. The round granules were distinctly larger in the mature birds, and there was a clear increase in the amount of intercellular material compared to immature chickens.

TEM showed that the well known A and B cells in synovial membrane could be clearly distinguished in both immature and mature birds. The more abundant B cells, which correspond to the smooth-surface elongated cells seen under SEM, were spindle-shaped and contained the usual organelles, much rough endoplasmic reticulum, prominent Golgi apparatus and a moderate number of mitochondria.

Small lipid droplets and filaments of indeterminate length with a diameter of approximately 10 nm were more abundant in mature than immature birds. Secretory vesicles measuring 150 to 240 nm were few in number and were seen in mature specimens only.

The basically round A cells were characterised by a large number of long filopodia extending in all directions, an abundance of large vacuoles 0.275 to 0.350 nm in diameter, small vesicles, moderate numbers of mitochondria, what looked like primary lysosomes 0.14 to 0.175 nm in diameter, a prominent Golgi apparatus and sparse rough reticulum (Fig. 9).



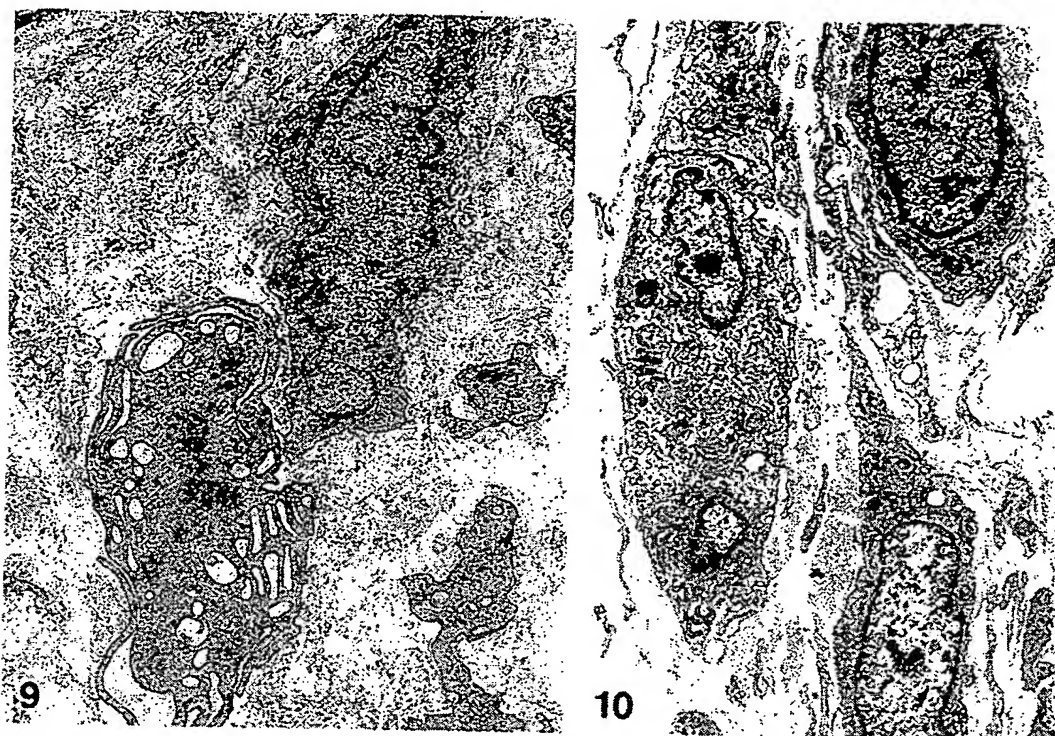


Fig. 9. TEM of lining cells. A cell with smooth-walled vacuoles, mitochondria, filopodia and scanty rough endoplasmic reticulum.  $\times 4070$

Fig. 10. B cells showing well developed rough endoplasmic reticulum and small vesicles. The matrix with collagen fibres covers the surface of the lining cells.  $\times 5000$

The cells did not differ in appearance in immature or mature birds, but they were more plentiful in mature than immature specimens. The location of A cells underwent pronounced changes with age. In immature chickens these cells were relatively sparse and restricted to the deepest layers of the synovial membrane, so that its surface was made up of B cells only (Fig. 10). This is in contrast to mature birds in whom the A cells were found in all layers, some being superficial and in contact with the articular cavity.

A basal lamina was not detected in the region of the synovial membrane. In addition to the A and B cells, some mast cells were seen in all layers, sometimes bordering on the articular cavity. Fibroblasts were restricted to the subsynovial layer.

## Discussion

Our study shows that that structure of the cartilage of the chicken knee joint differs in a number of ways from the corresponding cartilage in mammals.

Light microscopy demonstrates that in addition to the presence of blood vessels the most conspicuous difference was the regular occurrence of unmasked collagen fibres in chicken cartilage. In human and mammalian cartilage the unmasking of collagen fibres is commonly regarded as a sign of ageing [4, 21, 28] or degeneration [2, 6, 11]. The presence of clearly visible collagen fibres in immature and mature specimens is against this interpretation in chickens.

There were also definite differences in the arrangement of the collagen fibres. In the hyaline cartilage of humans and other mammals, superficial fibres run parallel to the articular surface; a second deeper layer runs parallel to the surface for a short distance only, and then bends towards the deeper zones to form arcades which superficially merge with adjacent arcades, so that the fibres cross each other forming acute angles [6, 16, 21, 40]. In chickens, only some fibres are arranged in this way, and most form bundles which run in different directions. Therefore, the four zones described by

Beninghoff [6] were not seen in our specimens. Other authors are of the opinion that chicken and rat articular cartilage are basically similar in structure [12, 18, 20], but they distinguish between zones of typical hyaline cartilage and fibrous cartilage.

As a result of the disposition of the collagen fibres in chickens, the chondrocytes are diffusely arranged and lack the typical columnar pattern found in mammalian cartilage [6, 11, 21]. Another dissimilarity is that chondrocytes in chicken cartilage usually form nests of two, and not four or more as is the case in mammals [2, 35, 36].

SEM studies show that in immature chickens the surface of the articular cartilage is absolutely smooth, whereas in mammals ridges and undulations are seen [29, 32, 33]. In mature birds the surface does not show humps and pits [10, 13, 14, 29], or undulations [7, 11, 32] which are likely to represent preparation artefacts [16, 37]. Although we were able to avoid the occurrence of oval depressions at the bottom of which collagen fibres are seen in mammals [37], they occurred in mature and not in immature chickens. Whether or not these were artefacts in chickens remains to be elucidated.

In contrast to the oval zones, the polymorphous areas lacking a surface coat and revealing collagen fibres are interpreted as a sign of degeneration. Identical changes were demonstrated in human articular cartilage at various stages of osteoarthritis [2, 7, 11, 26, 33] and in guinea pigs which were subjected to enforced long-term running [37].

TEM reveals that chicken chondrocytes may contain large amounts of intracytoplasmic filaments, especially in mature birds. This is clearly in contrast to mammals [5, 31] in whom the fibrils have been interpreted as signs of ageing and/or degeneration [5, 21, 35]. Although in our specimens the fibrils increase with age, their presence in immature animals contradicts the idea that they represent regressive changes.

In view of all these features it is evident that chicken cartilage is neither a typical hyaline or a typical fibrous cartilage. It is structurally more closely related to fibrous cartilage, but functionally closer to hyaline cartilage; we are inclined to call it fibrous hyaline cartilage for the present.

The ultrastructure of chicken synovial membrane conforms to that in mammals. Both A and B cells have been described previously [3, 9, 22, 24, 25, 30, 38, 39]. The so-called intermediate cells, which are intermediate in appearance between A and B cells [3, 23, 24] were not seen in chickens. The lack of intermediate cells has also been noted in rat synovial membrane [15].

A basal lamina demarcating intima from the sub-synovial layer has been reported [22], but we did not find this in our specimens. The absence of a basal lamina in human synovium has been recognised [3].

We think therefore that it is not correct to use chickens for research into experimental osteoarthritis because of these anatomical differences.

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